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L3: Entry 1 of 2 File: USPT Feb 11, 1997

DOCUMENT-IDENTIFIER: US 5601823 A
TITLE: Avian antitoxins to clostridium difficle toxin A

Brief Summary Text (4):
The genus Clostridium is comprised of gram-positive, anaerobic, spore-forming bacilli. The natural habitat of these organisms is the environment and the intestinal tracts of humans and other animals. Indeed, clostridia are ubiquitous; they are commonly found in soil, dust, sewage, marine sediments, decaying vegetation, and mud. [See e.g., P. H. A. Sneath et al., "Clostridium," Bergey's Manual.RTM. of Systematic Bacteriology, Vol. 2, pp. 1141-1200, Williams & Wilkins (1986).] Despite the identification of approximately 100 species of Clostridium, only a small number have been recognized as relatively common etiologic agents of medical and veterinary importance. Nonetheless, some of these species are associated with very serious diseases, including botulism, tetanus, anaerobic cellulitis, gas gangrene, bacteremia, pseudomembranous colitis, and clostridial gastroenteritis. Table 1 lists some of the species of medical and veterinary importance and the diseases with which they are associated. As virtually all of these species have been isolated from fecal samples of apparently healthy persons, some of these isolates may be transient, rather than permanent residents of the colonic flora. Nonetheless, as indicated in Table 1, the majority of these organisms may be associated with serious and/or debilitating disease. In most cases, the pathogenicity of these organisms is related to the release of

Brief Summary Text (6):
Perhaps because of their significance for human and veterinary medicine, much research has been conducted on these toxins, in particular those of C. botulinum and C. difficile.

Brief Summary Text (7):
C. botulinum

Brief Summary Text (8):
Several strains of Clostridium botulinum produce toxins of significance to human and and animal health. [C. L. Hatheway, Clin. Microbiol. Rev. 3:66-98 (1990).] The effects of these toxins range from diarrheal diseases that can cause destruction of the colon, to paralytic effects that can cause death. Particularly at risk for developing clostridial diseases are neonates and humans and animals in poor health (e.g., those suffering from diseases associated with old age or immunodeficiency diseases).

Brief Summary Text (9):
Clostridium botulinum produces the most poisonous biological toxin known. The lethal lethal human dose is a mere 10.sup.-9 mg/kg bodyweight for toxin in the bloodstream. Botulinal toxin blocks nerve transmission to the muscles, resulting in flaccid paralysis. When the toxin reaches airway and respiratory muscles, it results in respiratory failure that can cause death. [S. Arnon, J. Infect. Dis. 154:201-206 (1986).]

Brief Summary Text (10):
C. botulinum spores are carried by dust and are found on vegetables taken from the soil, on fresh fruits, and on agricultural products such as honey. Under conditions favorable to the organism, the spores germinate to vegetative cells which produces toxin. [S. Arnon, Ann. Rev. Med. 31:541 (1980).]

Brief Summary Text (11):
Botulism disease may be grouped into three types, based on the method of introduction of toxin into the bloodstream. Food-borne botulism results from ingesting improperly preserved and inadequately heated food that contains botulinal toxin. There were 355 cases of food-borne botulism in the United States between 1976 and 1984. [K. L. MacDonald et al., Am. J. Epidemiol. 124:794 (1986).] The death rate due to botulinal toxin is 12% and can be higher in particular risk groups. [C. O. Tacket et al., Am. J. Med. 76:794 (1984).] Wound-induced botulism results from C. botulinum penetrating traumatized tissue and producing toxin that is is absorbed into the bloodstream. Since 1950, thirty cases of wound botulism have been reported. [M. N. Swartz, "Anaerobic Spore-Forming Bacilli: The Clostridia," pp. 633-646, in B. D. Davis et al.,(eds.), Microbiology, 4th edition, J. B. Lippincott Co. (1990).] Infectious infant botulism results from C. botulinum colonization of the infant intestine with production of toxin and its absorption into the bloodstream. It is likely that the bacterium gains entry when spores are ingested and subsequently germinate. [S. Arnon, J. Infect. Dis. 154:201 (1986).] There have been 500 cases reported since it was first recognized in 1976. [M. N. Swartz, supra.]

Brief Summary Text (12):
Infant botulism strikes infants who are three weeks to eleven months old (greater than 90% of the cases are infants less than six months). [S. Arnon, J. Infect. Dis. 154:201 (1986).] It is believed that infants are susceptible, due, in large part, to the absence of the full adult complement of intestinal microflora. The benign microflora present in the adult intestine provide an acidic environment that is not favorable to colonization by C. botulinum. Infants begin life with a sterile intestine which is gradually colonized by microflora. Because of the limited microflora present in early infancy, the intestinal environment is not as acidic, allowing for C. botulinum spore germination, growth, and toxin production. In this regard, some adults who have undergone antibiotic therapy which alters intestinal microflora become more susceptible to botulism.

Brief Summary Text (13):
An additional factor accounting for infant susceptibility to infectious botulism is the immaturity of the infant immune system. The mature immune system is sensitized to bacterial antigens and produces protective antibodies. Secretory IgA produced in the adult intestine has the ability to agglutinate vegetative cells of C botulinum. [S. Arnon, J. Infect. Dis. 154:201 (1986).] Secretory IgA may also act by preventing intestinal bacteria and their products from crossing the cells of the intestine. [S. Arnon, Epidemiol. Rev. 3:45 (1981).] The infant immune system is not primed to do this.

Brief Summary Text (14):
Clinical symptoms of infant botulism range from mild paralysis, to moderate and severe paralysis requiring hospitalization, to fulminant paralysis, leading to sudden death. [S. Arnon, Epidemiol. Rev. 3:45 (1981).]

Brief Summary Text (15):
The chief therapy for severe infant botulism is ventilatory assistance using a mechanical respirator and concurrent elimination of toxin and bacteria using cathartics, enemas, and gastric lavage. There were 68 hospitalizations in California for infant botulism in a single year with a total cost of over $4 million million for treatment. [T. L. Frankovich and S. Arnon, West. J. Med. 154:103 (1991).]

Brief Summary Text (16):
Different strains of Clostridium botulinum each produce antigenically distinct toxin toxin designated by the letters A-G. Nearly all cases of infant botulism have been caused by bacteria producing either type A or type B toxin. (Exceptionally, one New Mexico case was caused by Clostridium botulinum producing type F toxin and another by Clostridium botulinum producing a type B-type F hybrid.) [S. Arnon, Epidemiol. Rev. 3:45 (1981).] Type C toxin affects waterfowl, type D toxin affects cattle, and type E toxin affects both humans and birds.

Brief Summary Text (18):
A heptavalent equine botulinal antitoxin which uses only the F(ab')2 portion of the antibody molecule has been tested by the United States Military. [M. Balady, USAMRDC Newsletter, p. 6 (1991).] This was raised against impure toxoids in those large animals and is not a high titer preparation.

Brief Summary Text (19):
A pentavalent human antitoxin has been collected from immunized human subjects for use as a treatment for infant botulism. The supply of this antitoxin is limited and cannot be expected to meet the needs of all individuals stricken with botulism disease. In addition, collection of human sera must involve screening out HIV and other potentially serious human pathogens. [P. J. Schwarz and S. S. Arnon, Western J. Med. 156:197 (1992).]

Brief Summary Text (20):
Infant botulism has been implicated as the cause of mortality in some cases of Sudden Infant Death Syndrome (SIDS, also known as crib death). SIDS is officially recognized as infant death that is sudden and unexpected and that remained unexplained despite complete post-mortem examination. The link of SIDS to infant botulism came when fecal or blood specimens taken at autopsy from SIDS infants were found to contain C. botulinum organisms and/or toxin in 3-4% of cases analyzed. [D. R. Peterson et al., Rev. Infect. Dis. 1:630 (1979).] In contrast, only 1 of 160 healthy infants (0.6%) had C. botulinum organisms in the feces and no botulinal toxin. (S. Arnon et al., Lancet, pp. 1273-76, Jun. 17, 1978.)

Brief Summary Text (22):
What is needed is an effective therapy against infant botulism that is free of dangerous side effects, is available in large supply at a reasonable price, and can be safely and gently delivered so that prophylactic application to infants is feasible.

Brief Summary Paragraph Table (1):
TABLE 1 _____ Clostridium Species of Medical and Veterinary Importance* Species Disease _____ C. aminovalericum Bacteriuria (pregnant women) C. argentinense Infected wounds; Bacteremia; Botulism; Infections of amniotic fluid C. baratii Infected war wounds; Peritonitis; Infectious processes of the eye, ear and prostate C. beijerinckikii Infected wounds C. bifermentans Infected wounds; Abscesses; Gas Gangrene; Bacteremia C. botulinum Food poisoning; Botulism (wound, food, infant) C. butyricum Urinary tract, lower respiratory tract, pleural cavity, and abdominal infections; Infected wounds; Abscesses; Bacteremia C. cadaveris Abscesses; Infected wounds C. carnis Soft tissue infections; Bacteremia C. chauvoei Blackleg C. clostridioforme Abdominal, cervical, scrotal, pleural, and other infections; Septicemia; Peritonitis; Appendicitis C. cochlearium Isolated from human disease processes, but role in disease unknown. C. difficile Antimicrobial-associated diarrhea; Pseudomembranous enterocolitis; Bacteremia; Pyogenic infections C. fallax Soft tissue infections C. ghnoii Soft tissue infections C. glycolicum Wound infections; Abscesses; Peritonitis C. hastiforme Infected war wounds; Bacteremia; Abscesses C. histolyticum Infected war wounds; Gas gangrene; Gingival plaque isolate C. indolis Gastrointestinal tract infections C. innocuum Gastrointestinal tract infections;

Empyema C. irregulare Penile lesions C. leptum Isolated from human disease processes, but role in disease unknown. C. limosum Bacteremia; Peritonitis; Pulmonary infections C. malenominatum Various infectious processes C. novyi Infected Infected wounds; Gas gangrene; Blackleg, Big head (ovine); Redwater disease (bovine) (bovine) C. oroticum Urinary tract infections; Rectal abscesses C. paraputrificum Bacteremia; Peritonitis; Infected wounds; Appendicitis C. perfringens Gas gangrene; Anaerobic cellulitis; Intra-abdominal abscesses; Soft tissue infections; Food poisoning; Necrotizing pneumonia; Empyema; Meningitis; Bacteremia; Uterine Infections; Enteritis necrotans; Lamb dysentery; Struck; Ovine Enterotoxemia C. putrefaciens Bacteriuria (Pregnant women with bacteremia) C. putrificum Abscesses; Infected wounds; Bacteremia C. ramosum Infections of the abdominal cavity, genital tract, lung, and biliary tract; Bacteremia C. sartagoforme Isolated from human disease processes, but role in disease unknown. C. septicum Gas gangrene; Bacteremia; Suppurative infections; Necrotizing enterocolitis; Braxy C. sordellii Gas gangrene; Wound infections; Penile lesions; Bacteremia; Abscesses; Abdominal and and vaginal infections C. sphenoides Appendicitis; Bacteremia; Bone and soft tissue infections; Intraperitoneal infections; Infected war wounds; Visceral gas gangrene; Renal abscesses C. sporogenes Gas gangrene; Bacteremia; Endocarditis; central nervous system and pleuropulmonary infections; Penile lesions; Infected war wounds; Other pyogenic infections C. subterminale Bacteremia; Empyema; Biliary tract, soft tissue and bone infections C. symbiosum Liver abscesses; Bacteremia; Infections resulting due to bowel flora C. tertium Gas gangrene; Appendicitis; Brain abscesses; abscesses; Intestinal tract and soft tissue infections; Infected war wounds; Periodontitis; Bacteremia C. tetani Tetanus; Infected gums and teeth; Corneal ulcerations; Mastoid and middle ear infections; Intraperitoneal infections; Tetanus neonatorum; Postpartum uterine infections; Soft tissue infections, especially related to trauma (including abrasions and lacerations); Infections related to use of contaminated needles C. thermosaccharolyticum Isolated from human disease processes, but role in disease unknown.

*Compiled from P. G. Engelkirk et al. "Classificiation", Principles and Practice of Clinical Anaerobic Bacteriology, pp. 22-23, Star Publishing Co., Belmont, CA (1992); (1992); J. Stephen and R. A. Petrowski, "Toxins Which Traverse Membranes and Deregulate Cells," in Bacterial Toxins, 2d ed., pp 66-67, American Society for Microbiology (1986); R. Berkow and A. J. Fletcher (eds.), "Bacterial Diseases," Merck Manual of Diagnosis and Therapy, 16th ed., pp. 116-126, Merck Research Laboratories, Rahway, N.J (1992); and O. H. Sigmund and C. M. Fraser (eds.), "Clostridial Infections," Merck Veterinary Manual, 5th ed., pp. 396-409, Merck & Co., Rahway, N.J. (1979).

Drawing Description Text (2):
FIG. 1 shows the reactivity of anti-C. botulinum IgY by Western blot.

Drawing Description Text (3):
FIG. 2 shows the IgY antibody titer to C. botulinum type A toxoid in eggs, measured by ELISA.

Drawing Description Text (28):
toxins from all Clostridium species are contemplated as immunogens. Examples of these toxins include the neuraminidase toxin of C. butyricum, C. sordellii toxins HT and LT, and the numerous C. perfringens toxins. In one preferred embodiment, toxins A, B, C, D, E, F, and G of C. botulinum are contemplated as immunogens. In another preferred embodiment, toxins A and B of C. difficile are contemplated as immunogens. Table 2 above lists various Clostridium species, their toxins and some antigens associated with disease.

Drawing Description Text (32):
In a preferred embodiment, the method of the present invention contemplates immunizing non-mammals with bacterial toxin(s). It is not intended that the present invention be limited to any particular toxin. In one embodiment, toxin from all clostridial bacteria sources (see Table 2) are contemplated as immunogens. Examples

of these toxins are C. butyricum neuraminidase toxin, C. difficile toxins A and B, C. perfringens toxins .alpha., .beta., .epsilon., and .iota., and C. sordellii toxins HT and LT. In a preferred embodiment, toxins A, B, C, D, E, F, and G from C. botulinum are contemplated as immunogens.

Drawing Description Paragraph Table (1):
TABLE 2 _____ Clostridial Toxins Organism Toxins and Disease-Associated Antigens _____ C. botulinum A, B, C.sub.1, C.sub.2, D, E, F, G C. butyricum Neuraminidase C. difficile A, B, Enterotoxin (not A nor B), Motility Altering Factor, Low Molecular Weight Toxin, Others C. perfringens .alpha., .beta., .epsilon., .iota., .gamma., .delta., .nu., .theta., .kappa., .lambda., .mu., .upsilon. C. sordelli/ HT, LT, .alpha., .beta., .gamma. C. bifermentans C. novyi .alpha., .beta., .gamma., .delta., .epsilon., .zeta., .nu., .theta. C. septicum .alpha., .beta., .gamma., .delta. C. histolyticum .alpha., .beta., .gamma., .delta., .epsilon. plus additional enzymes C. chauvoei .alpha., .beta., .gamma., .delta.

Detailed Description Text (36):
Production of C. botulinum Type A Antitoxin in Hens

Detailed Description Text (37):
In order to determine whether antibodies could be raised against the toxin produced by clostridial pathogens, which would be effective in treating clostridial diseases, antitoxin to C. botulinum type A toxin was produced. This example involves: (a) toxin modification; (b) immunization; (c) antitoxin collection; (d) antigenicity assessment; and (e) assay of antitoxin titer.

Detailed Description Text (39):
C. botulinum type A toxoid was obtained from B. R. DasGupta. From this, the active type A neurotoxin (M. W. approximately 150 kD) was purified to greater than 99% purity, according to published methods. [B. R. DasGupta & V. Sathyamoorthy, Toxicon, 22:415 (1984).] The neurotoxin was detoxified with formaldehyde according to published methods. [B. R. Singh & B. R. DasGupta, Toxicon, 27:403 (1989).]

Detailed Description Text (41):
C. botulinum toxoid for immunization was dissolved in PBS (1 mg/ml) and was enmlsified with an approximately equal volume of CFA (GIBCO) for initial immunization or IFA for booster immunization. On day zero, two white leghorn hens, obtained from local breeders, were each injected at multiple sites (intramuscular and subcutaneous) with 1 ml inactivated toxoid emulsified in 1 ml CFA. Subsequent booster immunizations were made according to the following schedule for day of injection and toxoid amount: days 14 and 21--0.5 mg; day 171--0.75 mg; days 394, 401, 409--0.25 mg. One hen received an additional booster of 0.150 mg on day 544.

Detailed Description Text (45):
Eggs were collected from day 409 through day 423 to assess whether the toxoid was sufficiently immunogenic to raise antibody. Eggs from the two hens were pooled and antibody was collected as described in the standard PEG protocol. [Example 1(c).] Antigenicity of the botulinal toxin was assessed on Western blots. The 150 kD detoxified type A neurotoxin and unmodified, toxic, 300 kD botulinal type A complex (toxin used for intragastric route administration for animal gut neutralization experiments; see Example 6) were separated on a SDS-polyacrylamide reducing gel. The Western blot technique was performed according to the method of Towbin. [H. Towbin et al., Proc. Nat'l Acad. Sci. U.S.A., 76:4350 (1979).] Ten .mu.g samples of C. botulinum complex and toxoid were dissolved in SDS reducing sample buffer (1% SDS, 0.5% 2-mercaptoethanol, 50 mM Tris, pH 6.8, 10% glycerol, 0.025% w/v bromphenol blue, 10% .beta.-mercaptoethanol), heated at 95.degree. C. for 10 min and separated on a 1 mm thick 5% SDS-polyacrylamide gel. [K. Weber and M. Osborn, "Proteins and Sodium Dodecyl Sulfate: Molecular Weight Determination on

Polyacrylamide Gels and Related Procedures," in The Proteins, 3d Edition (H. Neurath Neurath & R. L. Hill, eds), pp. 179-223, (Academic Press, N.Y., 1975).] Part of the gel was cut off and the proteins were stained with Coomassie Blue. The proteins in the remainder of the gel were transferred to nitrocellulose using the Milliblot-SDE electro-blotting system (Millipore) according to manufacturer's directions. The nitrocellulose was temporarily stained with 10% Ponceau S [S. B. Carroll and A. Laughon, "Production and Purification of Polyclonal Antibodies to the Foreign Segment of .beta.-galactosidase Fusion Proteins," in DNA Cloning: A Practical Approach, Vol. III, (D. Glover, ed.), pp. 89-111, IRL Press, Oxford, (1987)] to visualize the lanes, then destained by running a gentle stream of distilled water over the blot for several minutes. The nitrocellulose was immersed in PBS containing containing 3% BSA overnight at 4.degree. C. to block any remaining protein binding sites.

Detailed Description Text (46):
The blot was cut into strips and each strip was incubated with the appropriate primary antibody. The avian anti-C. botulinum antibodies [described in (c)] and pre-pre-immune chicken antibody (as control) were diluted 1:125 in PBS containing 1 mg/ml BSA for 2 hours at room temperature. The blots were washed with two changes each of large volumes of PBS, BBS-Tween and PBS, successively (10 min/wash). Goat anti-chicken IgG alkaline phosphatase conjugated secondary antibody (Fisher Biotech) was diluted 1:500 in PBS containing 1 mg/ml BSA and incubated with the blot for 2 hours at room temperature. The blots were washed with two changes each of large volumes of PBS and BBS-Tween, followed by one change of PBS and 0.1M Tris-HCl, pH 9.5. Blots were developed in freshly prepared alkaline phosphatase substrate buffer (100 .mu.g/ml nitroblue tetrazolium (Sigma), 50 .mu.g/ml 5-bromo-4-chloro-3-indolyl phosphate (Sigma), 5 mM MgCl.sub.2 in 50 mM Na.sub.2 CO.sub.3, pH 9.5).

Detailed Description Text (47):
The Western blots are shown in FIG. 1. The anti-C. botulinum IgY reacted to the toxoid to give a broad immunoreactive band at about 145-150 kD on the reducing gel. This toxoid is refractive to disulfide cleavage by reducing agents due to formalin crosslinking. The immune IgY reacted with the active toxin complex, a 97 kD C. botulinum type A heavy chain and a 53 kD light chain. The preimmune IgY was unreactive to the C. botulinum complex or toxoid in the Western blot.

Detailed Description Text (49):
The IgY antibody titer to C. botulinum type A toxoid of eggs harvested between day 409 and 423 evaluated by ELISA, was prepared as follows. Ninety-six-well Falcon Pro-bind plates were coated overnight at 4.degree. C. with 100 .mu.l/well toxoid [B. R. Singh & B. R. Das Gupta, Toxicon 27:403 (1989)] at 2.5 .mu.g/ml in PBS, pH 7.5 containing 0.005% thimerosal. The following day the wells were blocked with PBS containing 1% BSA for 1 hour at 37.degree. C. The IgY from immune or preimmune eggs was diluted in PBS containing 1% BSA and 0.05% Tween 20 and the plates were incubated for 1 hour at 37.degree. C. The plates were washed three times with PBS containing 0.05% Tween 20 and three times with PBS alone. Alkaline phosphatase-conjugated goat-anti-chicken IgG (Fisher Biotech) was diluted 1:750 in PBS containing 1% BSA and 0.05% Tween 20, added to the plates, and incubated 1 hour at 37.degree. C. The plates were washed as before, and p-nitrophenyl phosphate (Sigma) at 1 mg/ml in 0.05M Na.sub.2 CO.sub.3, pH 9.5, 10 mM MgCl.sub.2 was added.

Detailed Description Text (50):
The results are shown in FIG. 2. Chickens immunized with the toxoid generated high titers of antibody to the immunogen. Importantly, eggs from both immunized hens had significant anti-immunogen antibody titers as compared to preimmune control eggs. The anti-C. botulinum IgY possessed significant activity, to a dilution of 1:93,750 or greater.

Detailed Description Text (77):

In Vivo Neutralization of C. botulinum Type A Neurotoxin by Avian Antitoxin Antibody Antibody

Detailed Description Text (78):
This example demonstrated the ability of PEG-purified antitoxin, collected as described in Example 3, to neutralize the lethal effect of C. botulinum neurotoxin type A in mice. To determine the oral lethal dose (LD.sub.100) of toxin A, groups of BALB/c mice were given different doses of toxin per unit body weight (average body weight of 24 grams). For oral administration, toxin A complex, which contains the neurotoxin associated with other non-toxin proteins was used. This complex is markedly more toxic than purified neurotoxin when given by the oral route. [I. Ohishi et al., Infect. Immun., 16:106 (1977).] C. botulinum toxin type A complex, obtained from Eric Johnson (University Of Wisconsin, Madison) was 250 .mu.g/ml in 50 mM sodium citrate, pH 5.5, specific toxicity 3.times.10.sup.7 mouse LD.sub.50 /mg with parenteral administration. Approximately 40-50 ng/gm body weight was usually fatal within 48 hours in mice maintained on conventional food and water. When mice were given a diet ad libitum of only Enfamil.RTM., the concentration needed to produce lethality was approximately 2.5 times higher (125 ng/gm body weight). Botulinal toxin concentrations of approximately 200 ng/gm body weight were fatal in mice fed Enfamil.RTM. containing preimmune IgY (resuspended in Enfamil.RTM. at the original yolk volume).

Detailed Description Text (79):
The oral LD.sub.100 of C. botulinum toxin was also determined in mice that received known amounts of a mixture of preimmune IgY-Ensure.RTM. delivered orally through feeding needles. Using a 22 gauge feeding needle, mice were given 250 .mu.l each of a preimmune IgY-Ensure.RTM. mixture (preimmune IgY dissolved in 1/4 original yolk volume) 1 hour before and 1/2 hour and 5 hours after administering botulinal toxin. Toxin concentrations given orally ranged from approximately 12 to 312 ng/gm body weight (0.3 to 7.5 .mu.g per mouse). Botulinal toxin complex concentration of approximately 40 ng/gm body weight (1 .mu.g per mouse) was lethal in all mice in less than 36 hours.

Detailed Description Text (80):
Two groups of BALB/c mice, 10 per group, were each given orally a single dose of 1 .mu.g each of botulinal toxin complex in 100 .mu.l of 50 mM sodium citrate pH 5.5. 5.5. The mice received 250 .mu.l treatments of a mixture of either preimmune or immune IgY in Ensure.RTM. (1/4 original yolk volume) 1 hour before and 1/2 hour, 4 hours, and 8 hours after botulinal toxin administration. The mice received three treatments per day for two more days. The mice were observed for 96 hours. The survival and mortality are shown in Table 11.

Detailed Description Text (81):
All mice treated with the preimmune IgY-Ensure.RTM. mixture died within 46 hours post-toxin administration. The average time of death in the mice was 32 hours post toxin administration. Treatments of preimmune IgY-Ensure.RTM. mixture did not continue beyond 24 hours due to extensive paralysis of the mouth in mice of this group. In contrast, all ten mice treated with the immune anti-botulinal toxin IgY-Ensure.RTM. mixture survived past 96 hours. Only 4 mice in this group exhibited symptoms of botulism toxicity (two mice about 2 days after and two mice 4 days after after toxin administration). These mice eventually died 5 and 6 days later. Six of the mice in this immune group displayed no adverse effects to the toxin and remained alive and healthy long term. Thus, the avian anti-botulinal toxin antibody demonstrated very good protection from the lethal effects of the toxin in the experimental mice.

Detailed Description Paragraph Table (9):
TABLE 11 Neutralization of Botulinal Toxin A In Vivo

TOXIN DOSE ng/gm	ANTIBODY TYPE	NUMBER OF MICE ALIVE	NUMBER OF MICE DEAD
41.6	non-immune	0	10
41.6	anti-botulinal	10	

0 toxin _____

Other Reference Publication (8):
S. Arnon, "Infant Botulism: Anticipating the Second Decade," J. Infect. Dis. 154:201-206 (1986).

Other Reference Publication (9):
S. Arnon, "Infant Botulism," Ann. Rev. Med. 31:541 (1980).

Other Reference Publication (10):
K. L. MacDonald et al., "The Changing Epidemiology of Adult Botulism in the United States," Am. J. Epidemiol. 124:794 (1986).

Other Reference Publication (11):
C. O. Tacket et al., "Equine Antitoxin Use and Other Factors That Predict Outcome in Type A Foodborne Botulism," Am. J. Med. 76:794 (1984).

Other Reference Publication (13):
S. Arnon et al., "Infant Botulism: Epidemiology and Relation to Sudden Infant Death Syndrome," Epidemiol. Rev. 3:45 (1981).

Other Reference Publication (14):
T. L. Frankovich and S. Arnon, "Clinical Trial of Botulism Immune Globulin for Infant Botulism," West. J. Med. 154:103 (1991).

Other Reference Publication (15):
M. Balady, "Botulism Antitoxin Fielded for Operation Desert Storm," USAMRDC Newsletter, p. 6 (1991).

Other Reference Publication (16):
P. J. Schwarz and S. S. Arnon, "Botulism Immune Globulin for Infant Botulism Arrives-One Year and A Gulf War Later," Western J. Med. 156:197 (1992).

Other Reference Publication (17):
D. R. Peterson et al., "The Sudden Infant Death Syndrome and Infant Botulism," Rev. Infect. Dis. 1:630 (1979).

Other Reference Publication (18):
S. Arnon et al., "Intestinal Infection and Toxin Production by Clostridium Botulinum as One Cause of Sudden Infant Death Syndrome," Lancet, pp. 1273-1276, Jun. Jun. 17, 1978.

Other Reference Publication (42):
B. R. DasGupta & V. Sathyamoorthy, "Purification and Amino Acid Composition of Type A Botulinum Neurotoxin," Toxicon, 22:415 (1984).

Other Reference Publication (43):
B. R. Singh & B. R. DasGupta, "Molecular Differences Between Type A Botulinum Neurotoxin and Its Toxoid," Toxicon, 27:403 (1989).

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I. Ohishi et al., "Oral Toxicities of Clostridium botulinum Toxins in Response to Molecular Size," Infect. Immun., 16:106 (1977).

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